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Morphogenesis of *Escherichia coli*

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INTRODUCTION

The end result of *Escherichia coli* morphogenesis is a cylindrical tube with hemispherical caps. How does this shape come about? Shapes are not directly dependent on the chemical composition of the structure in question (157). For instance, *E. coli* cells treated with penicillin can have the same shape as glass tubes manipulated by a glass blower (reference 118 and references therein). Similarly, the alternating stretches of left- and right-handed helices between two fixed points are found not only in supercoiled DNA but also in the tendrils of a climbing plant while finding support at another twig (26). It would seem that shape has little to do with genes.

One single peptidoglycan macromolecule which has the shape of the cell (sacculus) can be purified from *E. coli* (172). This macromolecule represents a covalent fabric produced by gene-encoded enzymes. Of the many enzymes involved in its assembly, some assist in forming its lateral parts and others are specific for the hemispherical caps. Here is a clear division of labor. Where does gene expression end, and where does physics commence?

Of course, the sacculus is a kind of biochemical abstraction detached from its natural surroundings. In the growing cell, it is a highly dynamic structure where activities from different cellular compartments become integrated (122). It is a basic theme of this review that the transcriptionally active nucleoid and the cytoplasmic translation machinery form a structural continuity with the growing cellular envelope. This continuity is not static. After duplication of the genome, a new macromolecular fabric is organized to prepare the cell for division. What cellular events take place when new spherical caps are being formed? A discussion of the process leading to division also involves the topic of cellular polarity and intracellular architecture related to the external shape of the cell.

In outline, I will start with a physical model of the shape of *E. coli*. I will continue with its shape-maintaining layer, consider the cellular interior in relation to the cytoplasmic membrane, and then embark on division and polarity. Next, I will attempt to present an integrated view of the morphogenesis of *E. coli*, leaving perhaps more questions than answers. I will sometimes compare the situation in *E. coli* with that in higher organisms to underline the fact that prokaryotes and eukaryotes often have to solve similar problems (for instance, choosing the site of division) but also to emphasize the uniqueness of *E. coli.*

In the following sections, I will quote reviews relevant to the subject of the section in particular. However, besides these specialized papers, some bear more explicitly on the theme of this review because of the broadness of their approach (56, 57, 125, 129, 146, 147). I would also like to mention some books from which contributions have often been cited (22, 32, 46, 80, 124).

PHYSICAL ANALOG FOR THE SHAPE OF *E. COLI*

The idea that biological forms are subject to physical principles was elegantly illustrated many years ago by D'Arcy W. Thompson in his book *On Growth and Form*, which appeared in 1917. (I refer to the version abridged by J. T. Bonner in 1971 [157].) Little was then known about bacteria, and it is perhaps not surprising that *On Growth and Form in Bacteria* by Arthur Koch did not appear until 1995 (80). In recent years, he has developed a theory initially based on the physics of soap bubbles, which can account for the shape of various sphere- and rod-like bacteria (for reviews, see references 79 and 80). Thus, for a rod-shaped gram-negative bacterium like *E. coli* the comparison with cylindrical soap bubbles has been very helpful. To create a cylindrical soap bubble, two fixed rings are needed (Fig. 1). It is anticipated that the physical properties of the rings and the membrane of the bubble should somehow resemble those of the bacterial polar caps and lateral walls, respectively. Cylindrical soap bubbles spontaneously break in the center when their length reaches twice their radius multiplied by π, mimicking cell division (reference 79 and references therein). Again, how does this compare to the division of rod-shaped cells?

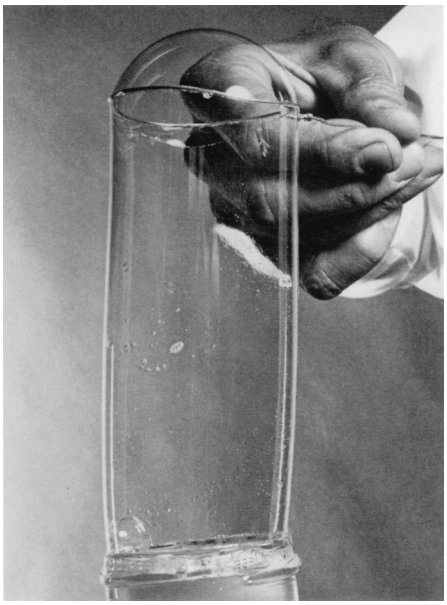

FIG. 1. A cylindrical soap bubble. Reprinted from reference 79 with permission of the publisher.

The physical parameters involved are hydrostatic pressure (P) and surface tension (T) in relation to cell shape. For a cylindrical structure, it has been shown that the radius (r) equals T/P (reference 79 and references therein). Thus, for *E. coli* it may be surmised that local reduction of T (at constant P) will result in division (81). However, not all rod-shaped bacteria are similar; for example, *E. coli* and *Bacillus subtilis* differ with respect to cell division. *E. coli* constricts, reducing the cell diameter, whereas *B. subtilis* forms a T-like structure (a septum), which splits upon completion. As a consequence, division in *E. coli* is subject to hydrostatic pressure but division in *B. subtilis* is not (79). The difference in division behavior between these gram-negative and gram-positive bacteria is related to the architecture and the mode of assembly of their cell walls. A monolayered peptidoglycan in the first case and a multilayered peptidoglycan in the second each exhibits its own way to grow and to maintain a constant thickness. In this review, of course, I will focus on *E. coli*. In a later section, I intend to return to the surface stress theory after having dealt with various aspects of the shape-maintaining macromolecular fabric of *E. coli.*

EXOSKELETON

Introduction

The envelope of *E. coli* contains three layers: the cytoplasmic membrane, the peptidoglycan or murein layer, and the outer membrane. The peptidoglycan layer resides between the cytoplasmic membrane and the outer membrane. There it is

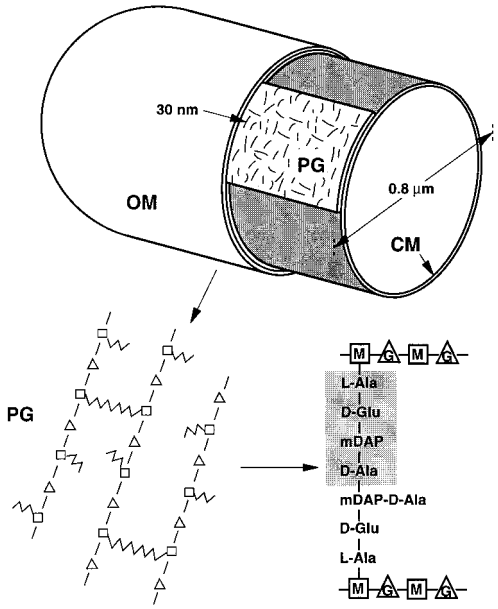

FIG. 2. Arrangement of glycan chains in the peptidoglycan layer and the schematic structure of peptidoglycan. CM, cytoplasmic membrane; G, *N*-acetylglucosamine; M, *N*-acetylmuramic acid; OM, outer membrane; PG, peptidoglycan. Modified from reference 122 with permission of the publisher.

embedded in a hydrated, largely proteinaceous substance, the periplasm. All the layers participate in shaping *E. coli*. However, early experiments showed that upon isolation, the covalently linked peptidoglycan layer retains the shape of the cell (172). It is most probably monolayered (89, 180), and it can rightly be described as an exoskeleton. This also justifies focusing on peptidoglycan when dealing with shape (69; for reviews also involving the other cell envelope layers, see references 105, 123, and 124).

Murein Biosynthesis

Peptidoglycan or murein is composed of glycan chains carrying peptide side chains (Fig. 2). These peptide side chains can interconnect the glycan chains via peptide bonds. A glycan chain is made up of disaccharide units (*N*-acetylglucosamine [GlcNAc]–*N*-acetylmuramic acid [MurNAc]). The sacculus grows due to the insertion of disaccharide pentapeptide units into the existing peptidoglycan with the aid of penicillin-binding proteins (PBPs) (for reviews, see references 5, 45, 66, 67, 68, 132, and 160). Synthesis of the disaccharide pentapeptide starts in the cytoplasm and ends in the cytoplasmic membrane.

In the cytoplasm, the five amino acids are added to UDP-MurNAc by specific enzymes (Fig. 3). The UDP-MurNAc-pentapeptide is then bound to undecaprenyl phosphate, forming a component called lipid I. Next, UDP-GlcNAc is added to lipid I, producing lipid II. The enzyme forming lipid I (translocase, MraY) contains several transmembrane segments, whereas the enzyme making lipid II (transferase, MurG) is associated with the inner face of the cytoplasmic membrane (for a review, see reference 161). The component (flippase?) exposing the prenylated disaccharide pentapeptide to the periplasm has not yet been identified.

In the periplasm (Fig. 3), a key role is played by the high-molecular-weight (HMW) PBPs: PBP1a, PBP1b, PBP2, and PBP3 (for a recent review, see reference 126). The first two enzymes are bifunctional in the sense that they connect the disaccharide pentapeptide to a glycan chain (transglycosylase activity) and that they link peptide side chains from neighboring glycan chains to each other (transpeptidase activity). PBP2 and PBP3 exhibit transpeptidase activity. Whether they can also carry out transglycosylation is not clear (126). It is generally assumed that PBP2 is involved in cell elongation and PBP3 is involved in cell division (see below).

Most genes coding for enzymes involved in the above sequence of reactions are clustered at the 2-min region of the bacterial chromosome (Fig. 4). This region also contains many important cell division genes, and for this reason it has been termed the dcw (division and cell wall) cluster (5). In the case of murein biosynthesis, there seems to be no strict correlation between the linear order of the genes on the chromosome and the sequence of reactions that their gene products are catalyzing to form the membrane-bound disaccharide pentapeptide (Fig. 3). Murein biosynthesis allows the rod-shaped *E. coli* cell to increase in length until division. How does the cell elongate?

Cell Elongation

Incorporation of the disaccharide pentapeptide into the existing sacculus takes place dispersively, i.e., over the whole surface of the lateral wall (for reviews, see references 80, 122, and 132). In other words, cell growth is not polarized as, for instance, in *Schizosaccharomyces pombe* (106). Impairment of PBP2, either by mecillinam or by growing a temperature-sensitive *pbpA* (the gene coding for PBP2) mutant at the nonpermissive temperature, leads to spherical cells; the same shape was observed in a *rodA* mutant. In temperature-sensitive *rodA* mutants, PBP2 activity is reduced when measured in a cell-free system (73). These authors have therefore suggested that PBP2 and RodA interact to maintain the rod shape. The enzymatic function of RodA is not known. However, it contains transmembrane sequences similar to the cell division protein FtsW (see below) and to SpoVE of *B. subtilis* (for a review, see reference 102).

Specific inhibition of PBP2 by the antibiotic mecillinam strongly reduces *meso*-[^{3}H]diaminopimelic acid ([^{3}H]DAP) incorporation into peptidoglycan. Inhibition of PBP1a and PBP1b by cefsoludin is much less severe, which has led to the idea that PBP1a and PBP1b provide primers for PBP2 to act upon (177). *E. coli* can also grow in the absence of a functional PBP2 when the intracellular level of ppGpp (guanosine tetraphosphate, the alarmone of the stringent response) is elevated (75). In this case, the cells are round, suggesting that the role of PBP2 has been taken over by PBP3. It is believed that spherical cells contain polar cap material (69).

It has long been recognized (173) that to enlarge a covalent structure, bonds have to be broken. Therefore, it would make sense to assume that sacculus enlargement is carried out by a multienzyme complex including lytic and synthetic activities. Does such a complex exist, and, if so, which proteins participate in it, either permanently or temporarily? Recent affinity chromatography studies suggest that such a bifunctional complex does exist (for a review, see reference 67). The composition of the complex is likely to be dynamic, and the presence of particular proteins would depend on the particular process being carried out, namely, cell elongation or cell division. A model (Fig. 5) has been suggested in which the hydrolysis of one glycan chain by a lytic activity (turnover) is accompanied by the simultaneous elongation of three new glycan strands (three-for-one model) (66, 67). Since non-cross-linked oligosaccharide precursors have not been detected so far (49), the

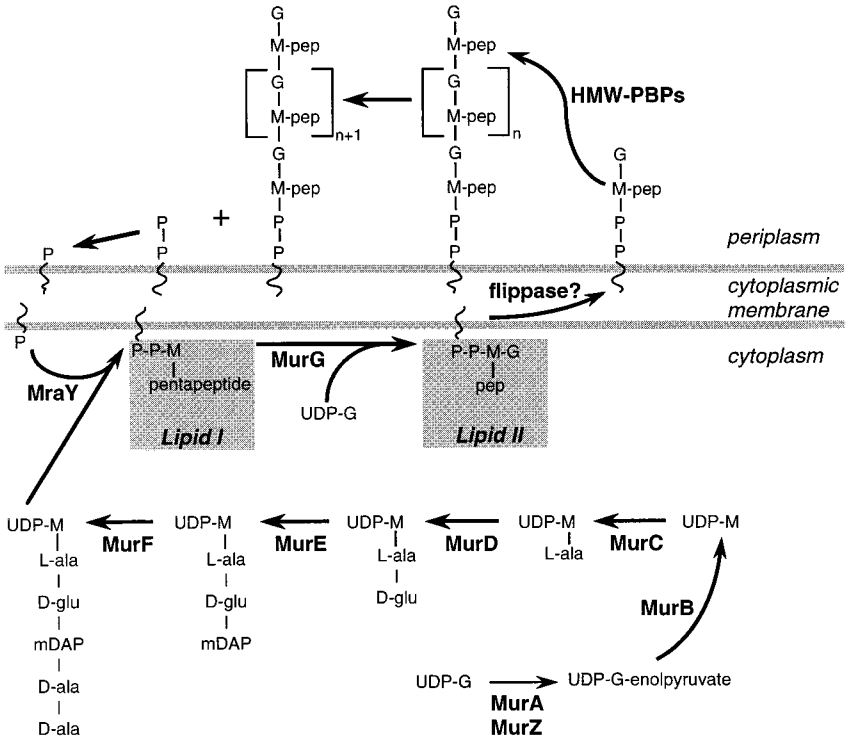

FIG. 3. Peptidoglycan assembly. The cytoplasmic steps leading to the prenylated disaccharide pentapeptide (lipid II) are shown. Through the action of a hypothetical flippase, the disaccharide pentapeptide becomes exposed to the periplasm. The membrane-bound disaccharide peptide becomes attached to a polymer that is also membrane bound. Glycan chain elongation occurs by transglycosylation. Elongation can occur at the nonreducing end of the glycan chain (this figure) or at the reducing end (not shown). G, *N*-acetylglucosamine; M, *N*-acetylmuramic acid; P, undecaprenylphosphate; PP, undecaprenyl biphosphate; pep, pentapeptide. Modified from reference 161 with permission from the American Society for Microbiology.

model implies that disaccharide pentapeptides are added one by one to each of the growing glycan chains. The model also illustrates the necessity of structural integration of lytic and synthetic activities. Once inserted, the glycan chains become oriented more or less perpendicular to the long axis of the cell (Fig. 2) (for reviews, see references 69, 80, and 122).

Analysis of peptidoglycan by high-pressure liquid chromatography has revealed a more complex chemical composition than was anticipated from earlier studies; in particular, many more muropeptides were found (31, 47, 66). In addition, several lytic enzymes have been identified (for a review, see reference 149). A puzzling situation is that all the lytic transglycosylase genes appear to be nonessential, at least under laboratory growth conditions (37). Presumably, the cell has arranged for backup measures to compensate for the loss of a particular gene.

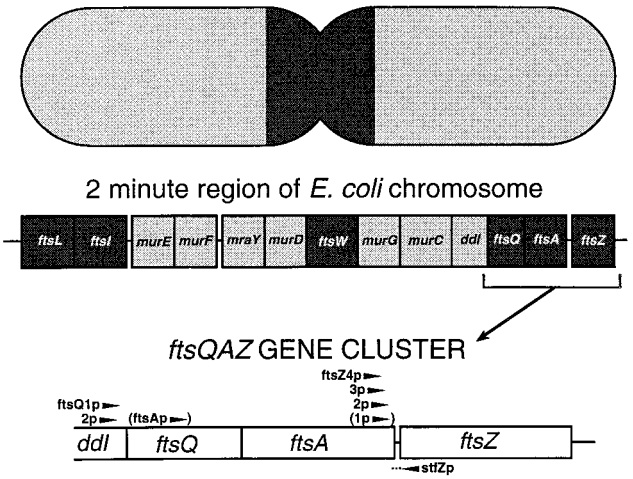

FIG. 4. Clustering of genes involved in cell division and cell wall synthesis (dcw cluster) in the 2-min region of the *E. coli* chromosome. Cell division genes are darkly shaded. The genes involved in the production of prenylated disaccharide pentapeptide (Fig. 3) are lightly shaded. The *ftsQAZ* gene cluster is depicted below. Note the position of the various promoters. Modified from reference 141 with permission of the publisher.

Biosynthesis of the sacculus is also accompanied by recycling of its murein. About 50% of the existing murein is reutilized per generation (48). A prominent peptidoglycan degradation product is a tripeptide, which is transported back into the cytoplasm by a permease. This component can be ligated as such to UDP-MurNAc by a murein peptide ligase (103). The ligase gene (*mpl*) resembles the *mru* genes coding for enzymes involved in assembly of the disaccharide pentapeptide unit, but it is found in a different gene cluster. The biological significance of recycling is not well understood. It has been speculated that the level of recycling intermediates is a reflection of the physiological condition of the cell (103). Clearly, in the overall context of peptidoglycan synthesis, turnover and recycling ensure that the exoskeleton is a highly dynamic structure.

Polar Cap Formation

During cellular division the lateral wall constricts in the center of the cell, eventually producing two hemispherical caps. At the leading edge of the ingrowing constriction, the cytoplasmic membrane and the nascent murein layer are tightly associated, as demonstrated by the local resistance to

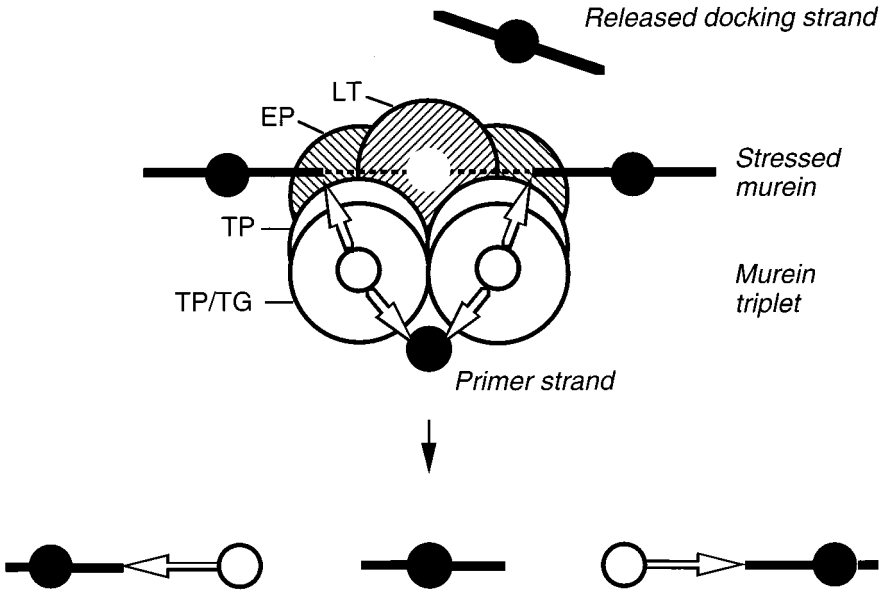

FIG. 5. Hypothetical periplasmic multienzyme complex involved in the final steps of peptidoglycan synthesis. The complex consists of enzymes with lytic and synthetic activities. Peptidoglycan is depicted in cross-section; black dots: glycan strands; horizontal lines: peptide cross-bridges. Lytic enzymes are endopeptidase (EP) and lytic transglycosylase (LT). Synthetic enzymes are bifunctional HMW PBPs with transpeptidase activity and transglycosylase activity (TP/TG); monofunctional transglycosylase (TG) and monofunctional transpeptidase (TP). The composition of the complex is dependent on the process being carried out, i.e., cell elongation or cell division. Some enzymes are in a dimeric form (see the text). Reprinted from reference 67 with permission of the publisher.

plasmolysis (96). On the basis of autoradiographic studies with the electron microscope on the incorporation of [^{3}H]DAP, it has been deduced that division-specific murein synthesis takes place at this leading edge with a gradient of activity spreading out over the polar caps (176). (This site coincides with the initial localization of the division protein FtsZ [see below].)

Many studies have been devoted to finding whether polar caps have a different chemical composition from that of the lateral wall. Since polar caps are rounded, one might perhaps expect (anthropomorphically) that they contain murein, which is multilayered and more cross-linked. To address this question, many approaches have been followed (for discussion, see references 80, 119, 121, 122, and 132). In all cases, no clear-cut chemical differences between longitudinal walls and polar caps have been observed, although glycan chain insertion appears to be single-stranded and multistranded, respectively as predicted by Koch in 1985 (for a review, see reference 122). All in all, one can infer that polar cap formation does not require peptidoglycan of a specific chemical composition. Of course it cannot be excluded that the differences are so subtle that they have not been detected by currently available techniques. In contrast, differences in the roles of HMW PBPs have been found. PBP3 (also called FtsI [Fts means filamenting temperature sensitive]) is involved exclusively in division, whereas PBP2, as indicated above, is involved in elongation of the cell (for reviews, see references 132, 150, 161, and 177). Impairment of PBP3 by using specific antibiotics like furazlocillin or cephalexin or by growing temperature-sensitive mutants at a nonpermissive temperature leads to filaments with unfinished, blunt (not sharp) constrictions. Therefore, it has been concluded that PBP3 is not involved in the onset of constriction but, rather, in its completion (also see below). As mentioned above, PBP2 and RodA seem to cooperate in cell elongation; similarly, PBP3 and FtsW may cooperate in the division process (for a review, see reference 102). However, recent observations revealed an *ftsZ*-like phenotype (filaments with no visible sign of constriction) for an *ftsW* mutant, suggesting that FtsW acts before PBP3 and possibly in combination with FtsZ (78).

From the foregoing, it appears that chemical specificity with respect to cell elongation and cell division resides primarily in the enzymes involved and not so much in the chemical structure of lateral walls and polar caps. In fact, peptidoglycan precursors in dividing cells are not different from those in nondividing cells (87). The above observations thus argue in favor of physical forces in shaping a cylinder with hemispherical caps.

Switch from Cell Elongation to Cell Division

Autoradiographic studies have also revealed that division-specific murein synthesis proceeds at the expense of lateral wall synthesis (for reviews, see references 119 and 122). Moreover, since PBP2 and PBP3 seem to be specifically involved in elongation and division, respectively (for reviews, see references 102, 150, and 177), this clearly indicates that a switch favoring one or the other PBP is present. In one view, the switch would be rather abrupt, with lateral wall extension excluding cell division and vice versa (91, 144). However, autoradiographic studies carried out with the electron microscope on [^{3}H]DAP incorporation do not support this idea (175, 181). Moreover, deeply constricted cells appear longer than slightly constricted ones (175), suggesting that cell elongation continues during division. How should one envision the switch? First, a signal has to be produced informing the cell that division is required. Such a signal may be related to the replication status of the genome and therefore will be an intracellular one. This signal might be distinct from a two-component system as used for the transduction of extracellular signals (unless a local difference in stress between the sacculus and cytoplasmic membrane, as postulated by Koch [80], or the presence of a so far unknown periplasmic component at the cell center [64a] functioned as an effector). Subsequently, an envelope associated

target in the cell center has to be constituted. This target is most probably the membrane component to which FtsZ binds (see below). Later, PBP3 has to be activated. An activation mechanism appears essential because PBP3 is continuously present during the cell cycle (178). Thus, many questions have already been posed. I will start with PBP3 activation and address the others below.

Several explanations can be invoked for the activation of PBP3. (i) Activation could be the result of the availability of a specific substrate at the right time. Thus, it has been suggested that GlcNAc-NAcMur tripeptides are the preferred substrate for PBP3 at division (7, 135). Such substrates could arise from periplasmic pentapeptides by removing the ultimate and penultimate D-alanines. Alternatively or in addition, they could arise by the specific production of a lipid II, containing a tripeptide chain (161) (Fig. 3). The first possibility, which requires a periplasmic carboxypeptidase I activity, seems unlikely because a triple deletion of PBP4, PBP5, and PBP6 is not lethal (38). However, deletion of these enzymes might be compensated by stimulating the activity of (unknown) related enzymes. Whether the carboxypeptidase II activity (producing a tripeptide from a tetrapeptide) is essential has not been tested because its gene has so far not been mapped (69). The second possibility has been investigated recently (162). In a cell-free system, it proved possible to synthesize lipid II containing a pentapeptide as well as the tripeptide, although in the latter case less material was made. In vitro, both lipid peptides were equally efficient as substrates for peptidoglycan synthesis with purified PBP1b; purified PBP3 showed no activity. Therefore, at present there is no evidence that lipid II tripeptide is a preferred substrate for PBP3. In fact, high-pressure liquid chromatography analysis of pulse-labeled murein in synchronized cells did not reveal an increase in the amount of radiolabeled tripeptide as breakdown products in dividing cells (31). It should, however, be borne in mind that PBP3 is most probably not involved in the earliest division step. Rather, we have invoked an initial penicillin-insensitive peptidoglycan synthesis system (PIPS) acting before PBP3 (for a review, see reference 119) (see below). In such a case, activation of PIPS could depend on the presence of a specific substrate. At present it is not clear which substrate that might be. (ii) As mentioned above, PBP3 is present throughout the cell cycle (178), with the ratio between the amounts of PBP3 and total cellular protein remaining constant. Consequently, the number of molecules of PBP3 increases as cells elongate. This can be interpreted to mean that a sufficient number of PBP3 molecules have to be present to allow the assembly of a functional division apparatus. Very recently, PBP3 has been localized to the cell center by immunofluorescence microscopy (174). Thus, PBP3 presumably becomes active through interaction with its neighbors in the divisome subassembly (see below). If this is so, activation by a specific substrate might not be necessary. I will return to the initiation of division after having discussed other proteins involved in cell division.

CYTOPLASMIC MEMBRANE AND CYTOPLASM

Cytoplasmic Membrane as a Structural Link between Cytoplasm and Wall

Animal cells. The aim of this section is to introduce the structural embedding of the cytoplasmic membrane in a cellular framework. To make this point clear, I will describe the membrane of an animal cell. In particular, the development of our knowledge of the erythrocyte membrane is illuminating. Originally, the erythrocyte membrane, like all other membranes, was visualized in the electron microscope as a triple-layered structure distinctly separated from other cellular structures. This image arose because the fixative used ($KMnO_4$) is quite destructive to all other cellular components. With the onset of better fixatives (glutaraldehyde) and especially because of the impressive emergence of fluorescent light microscopy, a completely different picture of cellular organization arose: the cytoskeleton came to the fore.

Nowadays, the plasma membrane of animal cells is considered an intermediate between the cytoskeleton and the extracellular matrix. The cytoskeleton is an all-pervading network of polymerized proteins, and the extracellular matrix is likewise constituted of organized polymers. Transmembrane protein dimers (integrins) serve to integrate the intracellular and extracellular systems. The remarkable observation that the two compartments show a comparable orientation of actin-containing microfilaments (inside) and of fibronectin (outside), as revealed by fluorescence light microscopy, underlines their structural continuity via the plasma membrane (focal adhesion sites) (4).

Bacteria. In the foregoing eukaryotic example, the plasma membrane functions as a structural intermediate between the extracellular matrix/cell wall and parts of the cytoskeleton. Can one extrapolate from this example to bacteria? The small size of bacteria makes them difficult objects for (fluorescence) microscopy. To further develop this point, it is essential to refer to the basics of prokaryotic cytology. As will be outlined below, the absence in prokaryotic cells of a nuclear membrane and of the endoplasmic reticulum (ER)-Golgi machinery, makes a direct structural link between cell envelope and its chromosome possible. Actually, transcription and translation, which over the course of evolution of eukaryotes became spatially separated, are not separated in bacteria. Protein transport in gram-negative bacteria implies protein export from the cytoplasm to the periplasm. Therefore, the bacterial periplasm is topologically equivalent to the ER lumen whereas the bacterial cell wall is equivalent to the eukaryotic extracellular matrix. Thus, the bacterial cytoplasmic membrane combines properties of the ER membrane and the eukaryotic plasma membrane.

Cytoplasmic membrane and cotranslational protein synthesis. How does one arrive at a more integrated view of the bacterial cytoplasmic membrane? During cellular growth, proteins with various functions have to be inserted into the cellular membrane, either permanently or transiently. For at least some proteins, evidence is available for cotranslational protein transport through the cytoplasmic membrane (160, 168). While in vitro studies have concentrated on posttranslational translocation of envelope proteins, it is likely that, in vivo, both co- and posttranslational translocation exists for many proteins (for reviews, see references 76, 114, and 158). Since bacterial transcription and translation are coupled (152), some functionally active parts of the nucleoid (encoding membrane or secreted proteins) are linked to the cytoplasmic membrane (Fig. 6) (52, 94, 115, 182).

Cytoplasmic membrane and cell wall. How is the cytoplasmic membrane associated with cell wall components, notably the murein layer? As mentioned above, murein assembly is carried out by the HMW PBPs. Typically, the HMW PBPs possess a short amino-terminal cytoplasmic tail, one transmembrane segment, and a large periplasmic domain. This topology makes it likely that nascent murein is functionally associated with integral membrane proteins (Fig. 5). Also, the end products of the cytoplasmic steps leading to the mono- and disaccharide pentapeptide are covalently linked to undecaprenyl phosphate in the cytoplasmic membrane (lipids I and II,

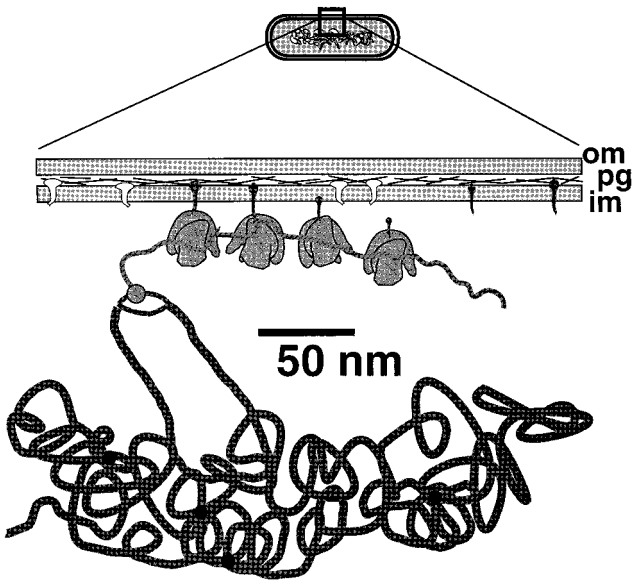

FIG. 6. Structural continuity between nucleoid and envelope through cotranscriptional biosynthesis of membrane proteins. Transmembrane proteins (white), involved in the synthesis of peptidoglycan, may restrict lateral diffusion of proteins being translocated (grey), and vice versa. im, inner or cytoplasmic membrane; om, outer membrane; pg, peptidoglycan. Modified from reference 182 with permission of the publisher.

respectively [Fig. 3]). Lipid II also serves as an intermediate for lipopolysaccharide synthesis (for a review, see reference 139); in addition, peptidoglycan synthesis and phospholipid synthesis appear tightly coupled (39, 132). This coupling most probably occurs at the level of the undecaprenyl phosphate-linked precursor (39).

The above presentation therefore shows that the cytoplasmic machinery of protein export results in cotranscriptional linkage of DNA to the cytoplasmic membrane (94), whereas at the same time the periplasmic machinery of murein synthesis is also linked to the cytoplasmic membrane (Fig. 6) (129).

I believe that there is sufficient evidence to make the point that the envelope and cytoplasm constitute a dynamically integrated structural entity. In other words, when bacterial shape is considered (admittedly most prominently reflected in the shape of its sacculus), the structural organization of the whole cell must be taken into account.

Does *E. coli* Have a Cytoskeleton?

Although the possibility of a fibrillar cytoplasmic network was recognized many years ago (145, 163), nowadays it seems to be generally agreed that *E. coli* does not contain a cytoskeleton. Eukaryotic structures like microfilament bundles, microtubules, and intermediate filaments have not been detected. If these structures had been present (in the above configurations), they would have been visualized by electron microscopy. The answer becomes different, however, if one considers a cytoskeleton to be a supportive structure to maintain cellular organization. Does *E. coli* have a supportive intracellular structure?

Because of its prokaryotic nature, transcription and translation are structurally coupled (104, 152). Electron microscopic autoradiography has shown that nascent RNA transcripts occur in the cytoplasm (142). Since ribosomes attach to these transcripts when they become available (152), bacterial cytoplasm can be conceived as a compartment filled with polyribosomes. Are polyribosomes which are producing cytoplasmic proteins only attached to the gene being transcribed? Or is there an underlying proteinaceous cytoplasmic matrix? Admittedly, in electron microscopic thin sections, there is little to be reliably discerned in the cytoplasm. One reason is that electron microscopic fixation and embedding procedures lead to considerable shrinkage and protein extraction, resulting in artifactual tight packing of ribosomes (184). Nevertheless, it appears relevant to discuss the possibility that elongation factor Tu (EF-Tu) and FtsZ are constituents of a primitive cytoskeleton.

Elongation factor Tu. Many years ago, Minkoff and Damadian (105) advocated the presence of an all-pervading actin-like cytoplasmic network (Fig. 7). This network was thought to be responsible for cellular contraction and swelling as related to potassium uptake. Other researchers at that time also collected evidence for the occurrence of actin (and myosin) in *E. coli* (115). Very recently, proteins that bound to rat brain anti-actin antibody, to phalloidin, and to DNase I were detected in *E. coli* (51). However, their genes have not yet been identified. Returning to the protein of Minkoff and Damadian (105), it later appeared to be EF-Tu, which bears only a limited similarity to actin (140). What remains interesting, however, is that EF-Tu can polymerize in vitro into threadlike filaments and elongated sheets (6) and that it is present in excess over ribosomes in the cell. About one-quarter of the roughly 70,000 EF-Tu molecules are thought to be active in protein synthesis (74), while the remainder might play a structural role in the cell (6, 74, 120). Bundles of EF-Tu polymers are not likely to occur in vivo, because they would have been observed in electron microscopic thin sections. On the other hand, if EF-Tu filaments consisting of linear arrays of monomers were present in the cytoplasm, we would not expect to visualize them by current electron microscopic techniques, while in the case of fluorescent labeling, the resolution might be too limited. There-

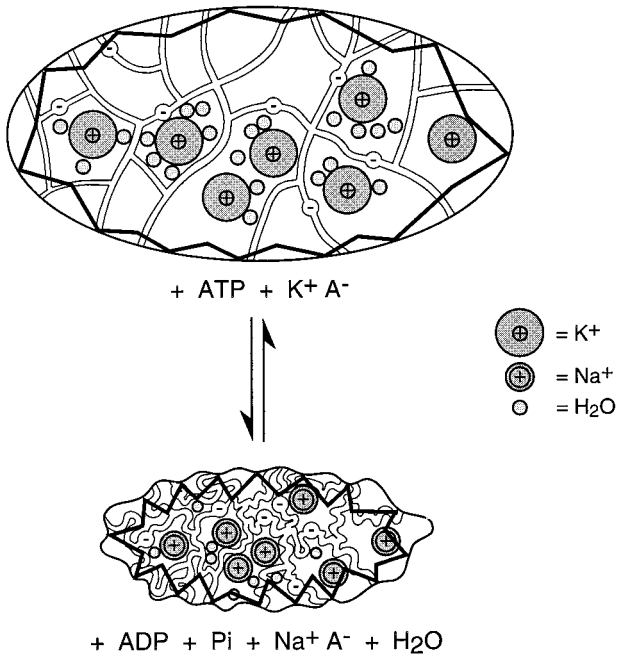

FIG. 7. Contractile proteins and the regulation of cell hydration in *E. coli*. Ion selectivity (Na^+ or K^+) depends on the cytoplasmic space available. Reprinted from reference 105 with permission from the American Society for Microbiology.

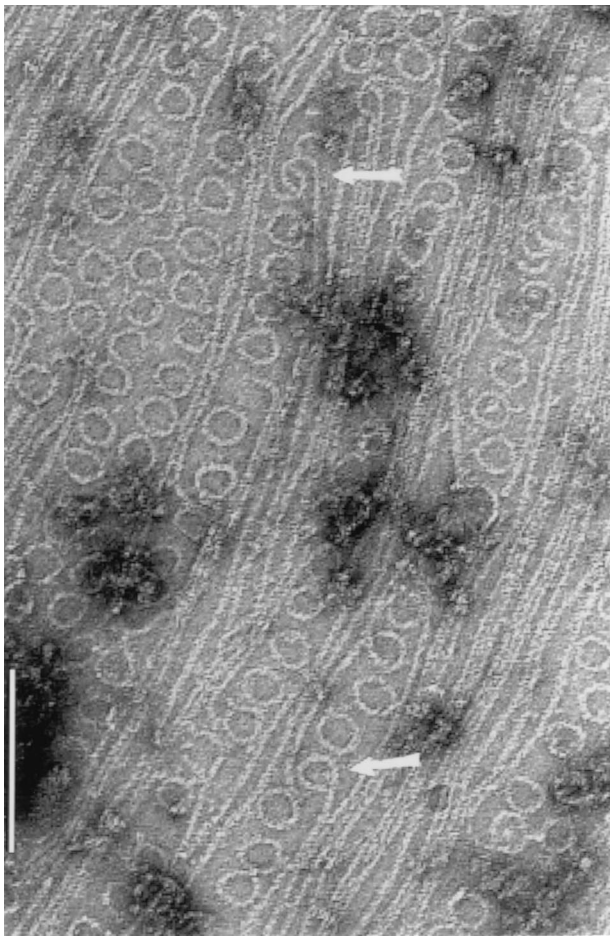

FIG. 8. In vitro assembly of FtsZ polymers and subsequent absorption to a polycationic lipid monolayer. Protofilaments (linear sequences of FtsZ monomers) occur singly or grouped into slender sheets. Rings have also formed. Reprinted from reference 40 with permission of the publisher.

fore, one has to conclude that the occurrence of EF-Tu filaments in *E. coli* remains a possibility.

Interestingly, in this context, the eukaryotic counterpart of EF-Tu, EF1a, also occurs in large excess in the cell. More strikingly, EF1a can bind to actin filaments and microtubules in vitro as well as in vivo (for a review, see reference 19). Actin filaments can be cross-linked by EF1a (131), whereas microtubules become destabilized (148). These observations indicate that EF1a affects cytoskeletal organization, presumably reflecting "compartmentalization of translation in eukaryotic cells" (19). In fact, the close association of polyribosomes with heart myofilaments had already been observed many years ago (16). It is difficult to accept that such compartmentalization is superfluous in a bacterium.

FtsZ. A stronger potential cytoskeletal candidate is the essential cell division protein FtsZ, which also occurs in large quantities (about 20,000 copies per cell) (11). FtsZ accumulates at the cell center, presumably in a polymerized form, to direct the division process. However, even during division, a substantial amount of FtsZ remains in the cytoplasm (166, 168). Given the polymerization potential of FtsZ (Fig. 8) (13, 40, 109), one might surmise that FtsZ filaments could occur in the cytoplasm to function as a kind of supportive framework. Recently, the green fluorescent protein technique has been used to localize FtsZ in the cell. After overproduction, FtsZ-green fluorescent protein fusions became visible as spirals in the cytoplasm (95). Whether these structures are aggregates or polymers is not clear, nor whether FtsZ at wild-type levels can form spirals. However, it should be emphasized that if such elements do occur, they will be present most probably in the form of thin linear arrays of monomers (Fig. 8).

Even so, the picture which emerges from this discussion of FtsZ reminds one of the redistribution of microtubular and microfilament arrays during the eukaryotic cell cycle. The cellular distribution of FtsZ is not the same in dividing and nondividing cells; therefore, the principle of spatial redistribution of cytoskeletal elements seems to hold for *E. coli* too.

Cytoplasmic ultrastructure. How should one imagine the cytoplasmic structure beyond the ribosome? A helpful visualization is presented in Fig. 9. It depicts a 100-nm window of *E. coli* cytoplasm (50; see also Fig. 2 in reference 184). Ribosomes, mRNA, tRNAs, proteins, and double-stranded DNA have been drawn to scale and according to their estimated respective concentrations. The "empty" space between these macromolecules has been enlarged 10 times in the lower figure (Fig. 9b). Water molecules have been depicted as the predominant species, and some larger molecules or ions reside in between. No distinction has been made between bulk water and water of hydration (44). The postulated cytoplasm-supporting structure (see above) should then be conceived as filaments built from proteins of approximate sizes, as depicted in Fig. 9a. Somehow, structured multienzyme complexes have

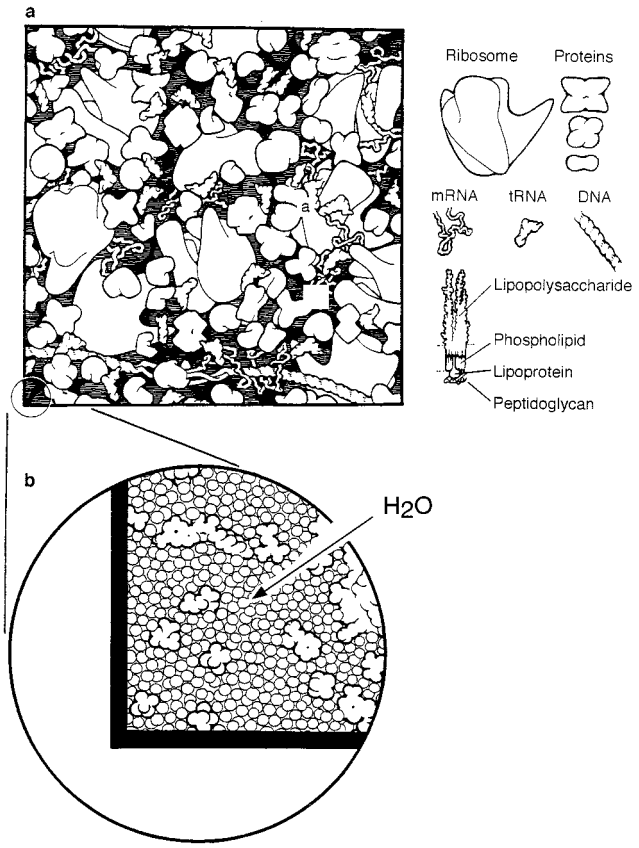

FIG. 9. (a) A 100-nm window of the *E. coli* cytoplasm. Ribosomes and other components have been drawn to scale. (b) A close-up of part of the window as indicated. Depicted are water molecules, some larger molecules, and part of a protein. Reprinted from reference 50 with permission of the publisher. See also reference 184.

to be fitted between the ribosomes and the putative filamentous elements (129, 151). Clearly, the degree of structural organization in the cell, ranging from ribosomes to water, is not known at present, mainly because adequate in vivo techniques are lacking. However, such a cytoplasmic "structure" involves the basic organization of living matter and therefore represents a major challenge for science to resolve.

DIVISOME STRUCTURE AND CELL DIVISION

Introduction

Cell division proteins. So far, we have concentrated on the periplasmic aspects of cell division, namely, the ingrowth of the peptidoglycan layer. The important demonstration by electron microscopic immunogold labeling that FtsZ assembles underneath the cytoplasmic membrane in the cell center (11) emphasizes the relevance of cytoplasmic events. FtsZ can bind GTP and possesses GTPase activity (11, 30, 140). In the presence of guanine nucleotides, FtsZ is able to polymerize in vitro, and various structures have been observed: sheets, protofilaments (a linear sequence of monomers), and rings (Fig. 8) (41). The putative GTP-binding site of FtsZ bears limited resemblance to eukaryotic tubulins (for reviews, see references 91 and 100). These properties, in conjunction with the characteristic localization of FtsZ during division, have led to the idea that polymerized FtsZ might function as a contractile cytoskeletal apparatus (for a review, see reference 93). Interestingly, the overall sequence of prokaryotic FtsZ resembles eukaryotic γ-tubulin more than it resembles α- or β-tubulin (100). γ-Tubulin occurs in spindle pole microtubules, which are specifically involved in the positioning of microfilaments in the division plane of animal cells (reference 42 and references therein).

Until now, I have mentioned two proteins that are specifically involved in cell division: PBP3 and FtsZ. Many others are known, and they are mostly designated Fts proteins (for reviews, see references 93 and 164). Little is known about the roles of most of them, although most are localized to the site of constriction. On the other hand, their cellular location clearly shows how they are distributed over three cellular compartments: cytoplasm, cytoplasmic membrane, and periplasm. Again, one is reminded of the structure of focal adhesions. The main bulk of many Fts proteins lies in the periplasm (near to the murein), while they are anchored in the cytoplasmic membrane: FtsI (PBP3), FtsL (53), FtsN (25), and FtsQ (15, 37). Others span the cytoplasmic membrane several times: FtsK (7) and FtsW (71). In addition to FtsZ, FtsA (136) and ZipA (54) are cytoplasmic division proteins.

Divisome and subassemblies. It might be expected that division proteins assemble into one multimeric structure, which carries out the division process. This structure has been called septalsome (64, 65), divisome (119), or septator (5). I prefer the term divisome, because in my view division proceeds through constriction and not through septation in *E. coli* (80, 123). How the various proteins spatially and temporally interact in the divisome is largely unknown. Very recently, FtsN (3) and PBP3 (174) have been localized at midcell. Genetic (159) and biochemical (121) evidence has been presented for the interaction of FtsA and PBP3. Indications have also been found for the interaction of FtsA and FtsZ (2, 95, 165), of PBP3 and FtsW (102), of PBP3 and PBP1b (67), and, finally, of FtsZ and ZipA (Z-interacting protein [54]). Furthermore, peptidoglycan hydrolases appear to bind to the HMW PBPs (67). In contrast to FtsZ, most division proteins occur in a limited number of copies per cell. For FtsA or FtsQ, for instance, there are not enough molecules to cover the circumference of the cell. Also, FtsA and FtsZ function at a certain ratio (24, 34), implying that the FtsZ ring is not saturated with cell division proteins. Therefore, as a tentative model, one can envision that the divisome is composed of subassemblies which are connected by FtsZ polymers (Fig. 10). Regarding the putative composition of a divisome subassembly (Fig. 11), it is important to realize that it has to carry out localized peptidoglycan synthesis. Therefore, it appears reasonable that MraY (producing lipid I) and MurG (producing lipid II) (Fig. 3), which provide disaccharide pentapeptides, should be considered essential components. Also included are hydrolytic enzymes (66) (Fig. 5). DNA loops (Fig. 6) are absent. Clearly, the structural connection between the cytoplasm and periplasm during division is quite different from the one during cell elongation.

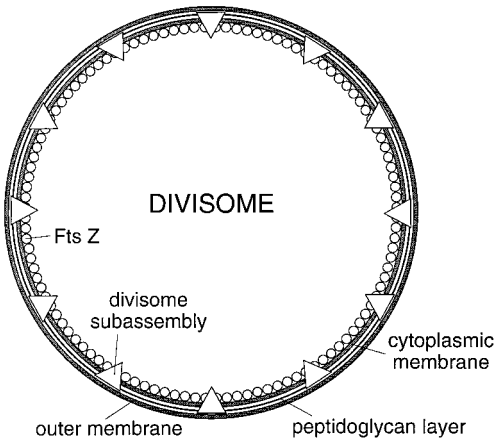

FIG. 10. Divisome composed of the FtsZ ring and divisome subassemblies.

Sequence of Events

Three distinct events. Microscopy of the filamentous phenotypes at the nonpermissive temperature has shown that *ftsZ* mutants possess nonindented lateral walls. In contrast, other *fts* mutants can initiate division, after which division becomes aborted as revealed by partial, blunt constrictions (8, 155). This has been interpreted to mean that FtsZ is the first division protein to become active and that the participation of other Fts proteins follows. In support of this conclusion, it has been

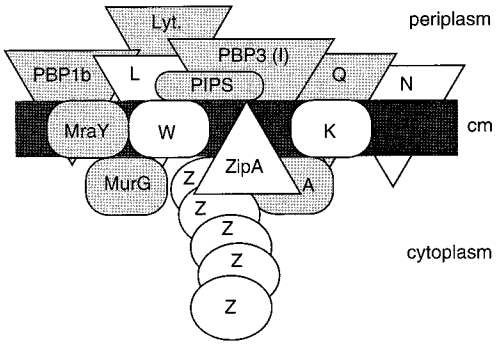

FIG. 11. Model of a divisome subassembly. Note that the structure unites the cytoplasm and periplasm. Although the association between individual pairs of some proteins has been demonstrated (see the text), the presumed multiple interactions have not yet been characterized. The gene products FtsA, FtsI (PBP3), FtsK, FtsL, FtsN, FtsQ, FtsW, and FtsZ have been denoted A, I, K, L, N, Q, W, and Z, respectively. Lyt. indicates that lytic enzymes must be present (see also Fig. 5); cm, cytoplasmic membrane.

shown recently, that the FtsZ ring can form at the nonpermissive temperature in *ftsA*, *ftsI* (*pbpB*), and *ftsQ* mutants (1). It has also been shown that FtsA can become localized to the cell center in the presence of nonfunctional PBP3 and FtsQ (2). Earlier data obtained by electron microscopic autoradiography (176) revealed that impairment of PBP3 did not inhibit the initiation of division, implying, in agreement with genetic evidence (8, 154), that this protein is involved in a later stage of division. For this reason, it has been argued that a PIPS step precedes the activation of PBP3 (119, 176). Although PIPS has not been characterized as yet, penicillin-insensitive enzymes acting on peptidoglycan have been described (119).

This brief survey suggests the following sequence of events. (i) FtsZ recognizes a specific target on the cytoplasmic membrane at the cell center and polymerizes there to form the FtsZ ring. Very recently, the possibility has been raised that the target is ZipA (54), although it now appears that ZipA ring formation is dependent on FtsZ (26a). (ii) Penicillin-insensitive peptidoglycan synthesis is activated. (iii) PBP3, FtsA, and FtsN associate with the ring (FtsK, FtsL, FtsQ, and FtsW might also become associated at this stage). (iv) Division progresses while peptidoglycan synthesis is carried out by PBP3 and other members (Fig. 5) of the multienzyme complex (the enzymatic functions of FtsK, FtsL, FtsN, FtsQ, and FtsW are not yet known). According to the scheme in Fig. 10, FtsZ would play a triple role during division: first, to establish the ring, second, to switch on PIPS, and third, to connect the divisome subassemblies. It is during the last stage that PBP3 and its companions are presumed to become active.

Do FtsA and FtsQ play a role in peptidoglycan synthesis? Thus far, I have emphasized the interaction between cell division gene products and the peptidoglycan-synthesizing system. In the divisome, FtsZ occupies a dominant position. However, archaebacteria such as the halobacteria do not contain peptidoglycan, yet they employ FtsZ (100, 170). These species have S-layer glycoproteins (containing glycan strands) superimposed on their cytoplasmic membrane and, not unexpectedly, lack β-lactamases (101). Like mycoplasmas, these organisms might also lack *ftsA* and *ftsQ* genes (43, 170) (remember also that *ftsQ*, *ftsA*, and *ftsZ* form one transcriptional unit in *E. coli* [Fig. 4]). Probably, therefore, FtsA and FtsQ play a role in peptidoglycan synthesis (170). Some indications support this idea. Overproduction of FtsA leads to bulging cells, presumably because of local increase in peptidoglycan synthesis (169). It might also be speculated that membrane-bound FtsA (143) interacts with MurG and or MraY (Fig. 3 and 11). Regarding *E. coli* FtsQ, the SXXK and SXN sequences characteristic of some PBPs and β-lactamases (45) are present in this protein (14), also suggesting a role in peptidoglycan assembly. However, what argues against this suggestion is that the above sequences could not be found in *Haemophilus influenzae*, an organism which closely resembles *E. coli.*

Motor proteins? As has been shown by electron microscopic autoradiography, [^{3}H]DAP-incorporation remained constant in three classes of dividing cells, grouped according to the degree of constriction. This has been interpreted to mean that "enzymes actively involved in murein synthesis become more and more closely packed" as division ensues (176). Consequently, during constriction, the divisome subassemblies are approaching each other, perhaps with the aid of motor proteins acting on FtsZ polymers (12). FtsA might be involved in movement of the divisome compartments, because it belongs to the actin family (143). Do motor proteins exist in *E. coli*? A 177-kDa motor-like protein (MukB [62]) has been implicated in nucleoid segregation. The overall organization of MukB resembles that of the so-called SMC proteins (133). These are thought to play a role in chromosome condensation, as required for mitosis. So far, there is no evidence for a role of motor proteins in the *E. coli* division process.

Division without DNA Partitioning

Apart from normal division at the cell center, division can, under certain conditions, also occur close to the cell poles to produce DNA-less minicells (for a review, see reference 29). Cell poles display their division capacity when the *min* locus is mutated or FtsZ is overproduced (171). The *min* locus contains three genes, *minC*, *minD*, and *minE* (28). Minicells arise by a normal division process, in the sense that the site of division shows increased [^{3}H]DAP incorporation (111) as well as FtsZ localization by immunogold labeling (12). There are two views on the mechanism of minicell formation, both of which are relevant for normal cell division. In one view (28), an inhibitory complex composed of MinC and MinD occupies polar potential division sites. The third protein, MinE, restricts the inhibitor from the cell center in wild-type cells (186). The possibility has also been raised that MinE directs the transport of the inhibitory complex MinCD to the cell poles (134). In both cases, MinE is involved in topological specificity, although it is not yet known in what way. Do Min proteins interact with FtsZ (10, 29)? In recent experiments, no direct interaction between FtsZ and MinCD could be detected (70). In fact, the model implies that MinC and MinD are capable of locating at three cytoplasmic membrane sites (two poles and the cell center) independently of FtsZ. The model would be experimentally supported, if labeled MinC at wild-type levels could be demonstrated specifically at those sites, but this has not so far been possible. When overproduced, MinD occurs everywhere underneath the cytoplasmic membrane (27).

In the alternative view of the role of Min proteins, attention has been focused on the fact that the minicell phenotype is accompanied by impairment of nucleoid partitioning (76, 113) and decreased negative supercoiling, resembling the effects of *gyrB* mutations (113). A possible explanation for minicell formation in this model is that impairment of DNA partitioning does not allow normal cell division to proceed (nucleoid occlusion [110, 183]). Thus, the topological-specificity role of MinE in this case might reside in transmitting a signal to the cell center whether the nucleoids have properly segregated or are on their way to doing so. In this latter model, the envelope has less autonomy in creating cytoplasmic domains for the assembly of the divisome.

MORPHOGENESIS

Polarity

To introduce the topic of polarity, I will first give some eukaryotic examples. Two organisms for which molecular genetics and morphological tools have been fruitfully used are *Drosophila melanogaster* and *Saccharomyces cerevisiae*. A size comparison between these organisms, a HeLa cell, and *E. coli* at once suggests differences in the degree of organizational complexity between eukaryotic cells and *E. coli* (Fig. 12).

Drosophila. Polarity in *Drosophila* centers around the establishment of its anteroposterior and dorsoventral axes. In the *Drosophila* egg, asymmetric distribution of some constituents is present from the outset. These are the follicle and nurse cells surrounding the egg, which determine its internal three-dimensional chemical topography. This can be visualized by the asymmetric distribution of mRNAs coding for anterior and posterior proteins, respectively. For instance, *bicoid* mRNA

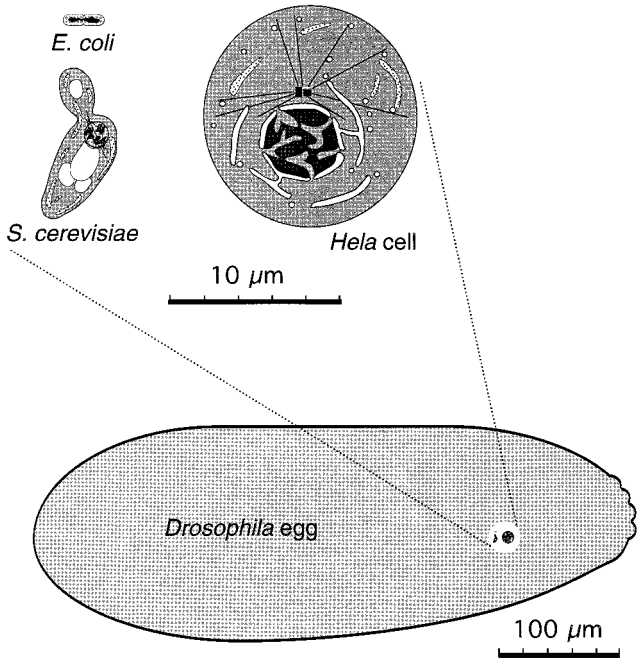

FIG. 12. Size comparison of a *Drosophila* oocyte containing a HeLa cell, a budding yeast, and a bacterium.

(produced by nurse cells) becomes concentrated at the anterior end of the egg, establishing a morphogen gradient (for a review, see reference 4). There does not seem to be an intracellular organizing agent that establishes the polarized distribution of the constituents of the embryo. Obviously, the early embryo contains topological memory due to its descent. Does this also apply to the yeast cell?

Saccharomyces cerevisiae. Polarity in *S. cerevisiae* research has focused on the selection of a new budding site (for a review, see reference 18). During this process, gene products have to find their targets (other gene products), which are located at the cellular membrane. How is a new bud site chosen?

Haploid cells choose a new bud site next to remnants of the previous division (axial bud site selection [reference 18 and references therein]). One of the several proteins involved in site selection is Bud10p, a transmembrane protein. Interestingly it bears some resemblance to integrins of animal cells (55) (integrins connect the cytoskeleton with the extracellular matrix).

In diploids, a bud may arise opposite the previous division site. Such a specific location implies in itself an established polarity. In fact, division remnants might play a role here too. It is conceivable that part of the division remnant has been carried to the pole of the newly formed bud to provide recognition points for proteins involved in budding. Thus, both in haploids and in diploids, remnants of the previous division are used to establish a new bud site.

Bacteria. How does a prokaryotic cell compare to the above scheme? Well-known research themes are sporulation in *Bacillus* spp. and the development of specialized cells in *Caulobacter.*

In *Bacillus*, spores tend to develop near the oldest pole (reference 63 and references therein), although the spore does not seem to have a preference for the old or the new chromosome (86). In fact, immunofluorescent staining of FtsZ has revealed that upon entry into sporulation, midcell localization of FtsZ switches to a bipolar pattern (90). Eventually, septation occurs at one pole only. How does asymmetry arise, and what are its consequences? At present, it is not known by what mechanism the spore-related septum is chosen. The consequences of its asymmetric placement are clearer; this leads to differences in local activities of transcription factors. As a result, a spore develops and the mother cell dissolves (for a review, see reference 41).

In *Caulobacter*, cellular polarity is marked by the placement of polar appendages. A predivisional cell carries a flagellum at one end and a stalk at the other. The two cellular compartments also differ in gene expression, which has been shown to be related to preexisting cellular asymmetry (for a review, see reference 146). In these examples, polarity or asymmetry is quite apparent.

However, what about detecting polarity if easily discernible structures like spores and stalks do not occur? At the envelope level, at least three distinct cellular areas can be distinguished (23): the old pole, which is virtually inert with respect to [^{3}H]DAP incorporation (85), and the lateral wall and the newly forming polar cap, which are involved in [^{3}H]DAP incorporation (for reviews, see references 23 and 122). As outlined above, the cell wall is connected to cytoplasmic structures via the cytoplasmic membrane (Fig. 6). At the newest pole, these connections are clearly less tight, because it is there that plasmolysis takes place most readily (112). Thus, each new cell is born with preestablished polarity. At the DNA level, it has been found that semiconservatively replicated DNA strands do not distribute randomly over the daughter cells (for a review, see reference 23). The newest strand shows a tendency to be located in the cellular compartment near the newest pole (60). These observations further add to the notion of preexisting polarity in *E. coli*. Can further evidence of polarity (in the absence of cytological markers) be demonstrated?

Three recent *E. coli* examples (Fig. 13) arose through immunogold labeling of chemoreceptor proteins (99) and the cell division protein FtsZ (11) and fluorescence microscopy of ZipA (54). The first is localized at the poles, and the division proteins are found midway between the poles. To adopt these locations, target sites have to be there first, suggesting the occurrence of specific domains in the cytoplasmic membrane. It has been shown that in *E. coli*, complexes of chemoreceptor proteins and two cytoplasmic proteins (CheA and CheW) are localized predominantly to a cell pole (Fig. 13a) (99). Polar location appeared to depend on the combined presence of chemoreceptor and CheW. Together, they seem to recognize a target at the cytoplasmic membrane. How did the target arise? It has been speculated (146) that it represents a remnant from a previous division. If so, polarization in yeast and polarization in *E. coli* have the same underlying principle: polarization arises from polarization.

Selection of the Division Site

During cell division, FtsZ becomes localized to the cell center at the cytoplasmic membrane (Fig. 13b) (1, 11). In this figure, division has not yet started but the nucleoids have separated. It appears that an FtsZ-containing domain, probably involving ZipA (Fig. 13c), has arisen in the cytoplasmic membrane. How did such a domain arise?

Nucleoid partitioning and cell division. It seems almost trivial to state that division serves to distribute duplicated chromosomes to new daughter cells. It is therefore not a big step to presume that nucleoid partitioning and cell division are also temporally and structurally related in *E. coli*. Alternatively, is bacterial division autonomous?

Some time ago, it was stated that the nucleoid exerts a "veto power" (61) over cell division, meaning that the nonpartitioned

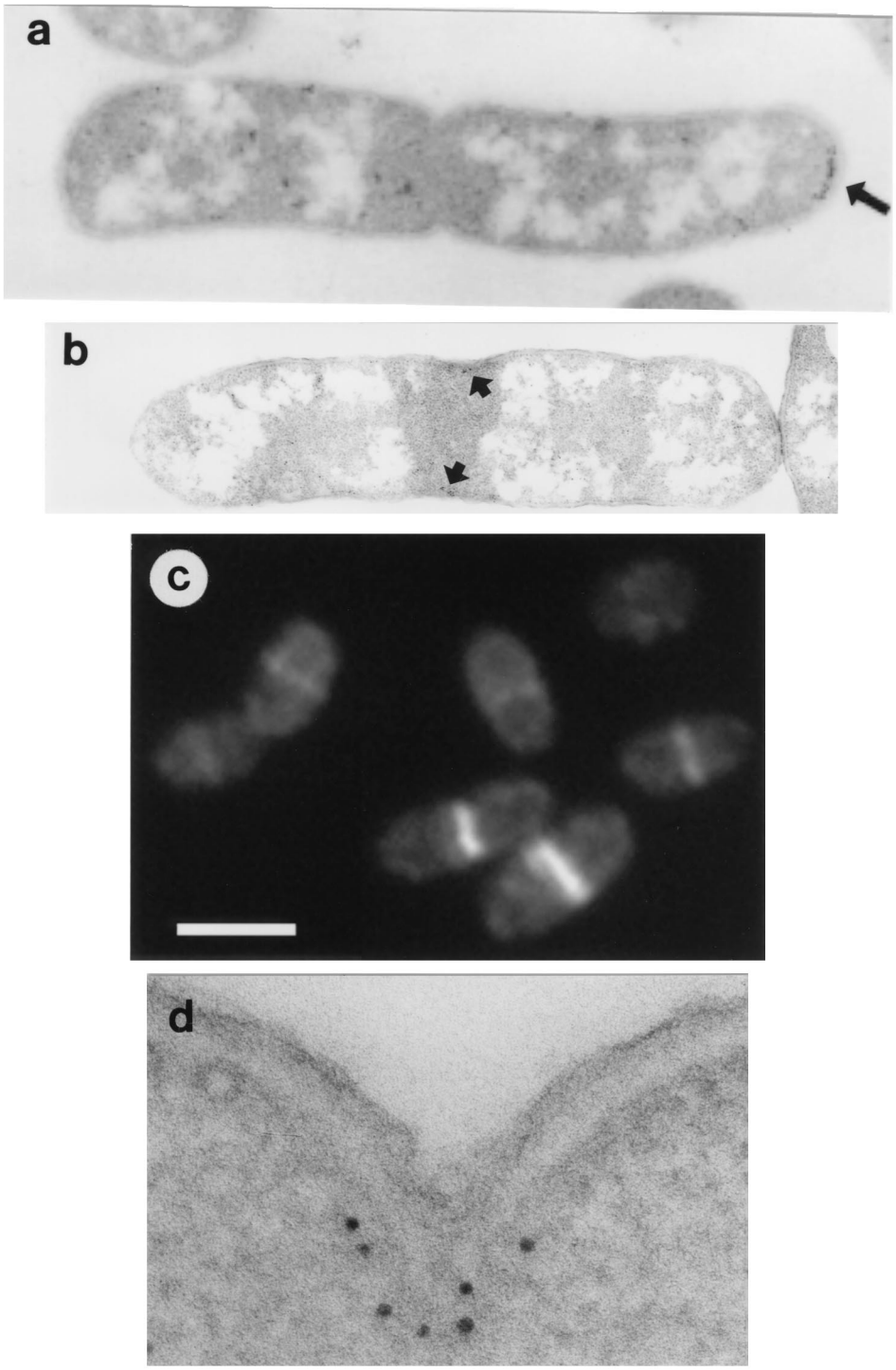

FIG. 13. (a) Polar localization (arrow) of an *E. coli* chemoreceptor (Tsr) as revealed by immunogold electron microscopy. Reprinted from reference 99 with permission of the publisher. (b) FtsZ localization at the cell center underneath the cytoplasmic membrane (arrows) at a nucleoid-free area, shown by immunogold electron microscopy. Reprinted from reference 11 with permission of the publisher. (c) ZipA ring as revealed by fluorescence of a protein fusion between ZipA and green fluorescent protein. Bar, 2 μm. Reprinted from reference 54 with permission of the publisher. (d) Immunogold labeling of FtsZ with monoclonal antibody MAb12 (165).

nucleoid represents a physical barrier for the division process (reference 183 and references therein). An experimental result underscoring this notion is shown in Fig. 14. It represents an autoradiographic experiment where the incorporation of [^{3}H]DAP into peptidoglycan was monitored with an electron microscope (111). Because the resolution of this technique is limited to about 100 nm (80), the experiment was executed with a *dnaX*(Ts) mutant, in which DNA replication stops im-

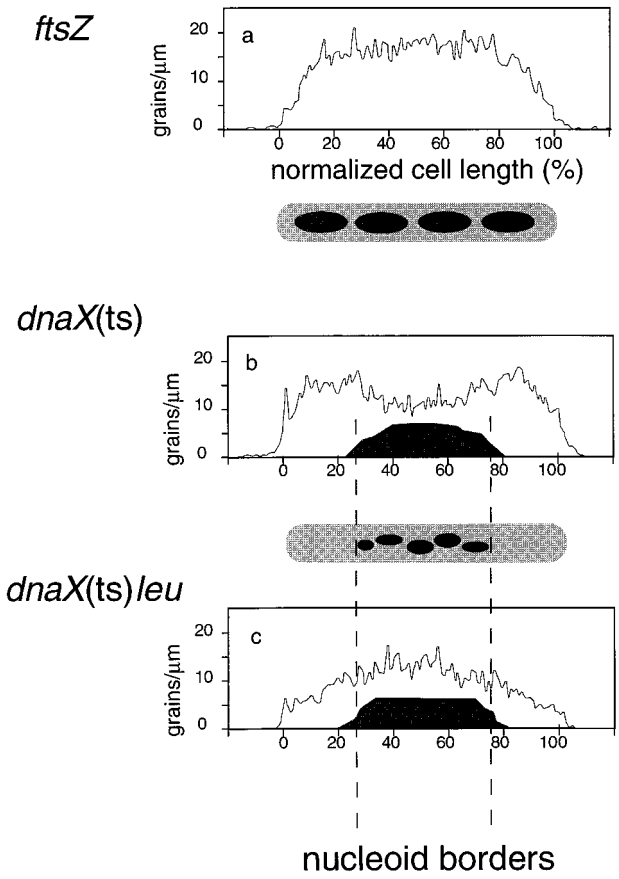

FIG. 14. Autoradiography of [^{3}H]DAP incorporation (a and b) and of [^{3}H]leucine incorporation (c). (a) Silver grain distribution over whole-mount *ftsZ* filaments. (b) Silver grain distribution over whole-mount *dnaX* filaments. (c) Silver grain distribution over *dnaX* filaments pulse-labeled with [^{3}H]leucine. The shaded areas in panels b and c represent the position of the nucleoid. Reprinted from reference 111 with permission of the publisher.

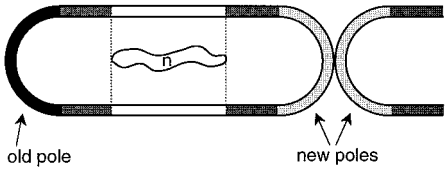

FIG. 15. Proposed envelope domains in *E. coli*: old pole, new pole, nucleoid-associated domain, and domain between pole and nucleoid-associated domain. n, nucleoid.

mediately at the nonpermissive temperature. Under these conditions, cells make filaments, and comparatively large DNA-less and DNA-containing areas can be distinguished. In other words, this makes it possible to compare [^{3}H]DAP incorporation into the envelope bordering DNA with envelope that does not. A striking result is that in the vicinity of the nucleoid, there is less [^{3}H]DAP incorporation than elsewhere (nucleoid occlusion [183]). Extrapolating this outcome to the much shorter wild-type cell, this suggests that after nucleoid separation, a circular envelope domain arises in the cell envelope, where [^{3}H]DAP incorporation is less strongly inhibited, allowing peptidoglycan synthesis to increase locally as needed for the onset of division. Clearly, this can happen only when the FtsZ ring has also been formed (Fig. 13b). How should one envision the reduction of peptidoglycan synthesis in the vicinity of the nucleoid? In an attempt to give an explanation, I will return to a previous section of this review.

Cytoplasmic membrane domains. It seems reasonable to suppose that cotranscriptional synthesis of polypeptides (94) occurs most prominently in the vicinity of the nucleoid. Actually, in the experiment documented in Fig. 14, [^{3}H]leucine incorporation was also measured (111). (It should be realized that in this experimental setup, no distinction could be made between synthesis of cytoplasm- and membrane-bound protein.) It was found that the incorporation of leucine was most intensive near the nucleoid. Therefore, I suggest that two extra envelope-associated domains should be added to the ones mentioned before. These putative domains (Fig. 15) are distinguished by the relative enrichment of cotranscriptional DNA/membrane structures on the one hand and the relative enrichment of membrane components of the peptidoglycan-synthesizing machinery on the other. It would seem that proteins in membranes are confined to discrete areas.

By using "optical tweezers," an impression could be gained about the trajectories that proteins can follow in an animal membrane (single-particle trapping). This has led to the concept of a membrane skeleton composed of domains (88). These domains have a size range of 0.1 to 1 μm, suggesting that a limited number would be present in *E. coli* (Fig. 1 in reference 86). However, it is not clear whether the factors that limit domain size in animal cells are the same as those in *E. coli.*

Nevertheless, I have tried to develop the notion that cytoplasmic membrane domains are topologically determined by the spatial position of the functionally active nucleoid.

Cytoplasmic domains? In the above, the focus has been on domains in the cytoplasmic membrane. What about the cytoplasm in relation to cell division? It has been assumed that DNA replication and peptidoglycan synthesis withdraw pyrimidine nucleotides from the same pool (153). If so, one can imagine that termination of DNA replication might result in a local surplus of deoxyribonucleotides at the cell center (183). Part of this could then be used to produce extra UDP-GlcNAc, the starting material for peptidoglycan synthesis (Fig. 3). However, elongating and dividing cells have the same levels of peptidoglycan precursors (87). Therefore, the idea can work only if the cell can provide for a local (compartmentalized) increase in murein precursors at the expense of murein precursors elsewhere (119).

Division site selection without invoking the nucleoid. Is bacterial cell division regulated at the envelope level? There are two views that favor this idea. The first is based on the observation that when plasmolysis is induced in growing cells, spaces or bays arise, in particular at a cell pole. However, a fraction of such bays tend to occur in the cell center in nondividing cells or at one-quarter and three-quarter of the cell length in dividing cells, i.e., at future division sites. Although morphologically distinct, the bays have been linked genealogically to so-called periseptal annuli (20). These are circumferential zones of adhesion between the cytoplasmic membrane and peptidoglycan layer that run completely around the cell cylinder, and the region between them defines the periseptal domain where constriction starts (20, 21). Such annuli become visible in the cell center upon plasmolysis of dividing cells, and they have been most clearly observed in a *Salmonella typhimurium lkyD* mutant, where cell separation is impaired (96). Does the occurrence of a plasmolysis bay at a future division site reflect a property related to cell division? As mentioned above, the first visible sign of incipient division in nonplasmolyzed cells is the positioning of FtsZ at and of ZipA in the cytoplasmic mem-

brane in the cell center. This implies that the property referred to above has no connection with FtsZ, because FtsZ and ZipA are not yet located to the future division site; i.e., only one ring is found in normally dividing cells (11). The genealogical relationship between plasmolysis bays and periseptal annuli has been questioned by Woldringh (180), and the controversy has not yet been settled (cf. MicroCorrespondence [1995] in *Mol. Microbiol.* **17:**597–599 and reference 130).

The second model (the central stress model) for the triggering of cell division on the cell envelope level (irrespective of the nucleoid) has been presented by Koch and Höltje (84). They assume differences in stress between the cytoplasmic membrane and the peptidoglycan layer. These differences are supposed to be related to the respective elastic properties of the two layers. Division occurs when "the threshold is locally exceeded" (80). This would result in conformational changes of proteins, thus providing for a signal that induces local wall growth in the middle of the cell. Although plausible, the model is difficult to test by present techniques.

The central-stress model (84) is not necessarily in conflict with the nucleoid occlusion model (183) as expanded here. The cytoplasmic domains depicted in Fig. 15 might well give rise to differential stresses along the lateral wall. What all models have in common is the differentiation of the cell envelope into domains.

Positioning of Gene Products

Protein-protein interaction. Much is known about protein-protein interactions for proteins which are diffusely inserted in the bacterial envelope and which are targeted to the cytoplasmic membrane, the periplasm, or the outer membrane. However, when considering division, we are dealing with a sharply localized target: the cell center. As outlined above, the composition of the cytoplasmic membrane might be different at the site of division, not only with respect to proteins but perhaps also with respect to phospholipid composition (80, 127, 128). A component X has been postulated as a connecting element between cytoplasm (FtsZ) and periplasm (peptidoglycan) (119). This protein, together with other components (lipids?), might create a specific membrane domain favorable for the binding of FtsZ and ZipA. Of course, the question of how ZipA arrives at the cell center is still open. Although PBP3 and FtsA can also associate with FtsZ, it is likely that this occurs at a later stage of the division process (see the previous section). It has also been suggested that cytoplasmic FtsZ has to change its conformation (exposing its hydrophobic segments) to bind to its cytoplasmic membrane target (165, 166). FtsA, which can bind ATP (143), could be instrumental in causing this change. However, the fact that the FtsZ-ring can form in the presence of a defective FtsA molecule (1) argues against such a role for FtsA. FtsA might reach FtsZ (while forming a divisome subassembly [Fig. 10]) by diffusion through the cytoplasm (however, see below). Transmembrane proteins like PBP3 and FtsQ might diffuse through the cytoplasmic membrane and become stuck when they meet ZipA and FtsZ. A similar mechanism has been suggested for the assembly of the chemoreceptor complex at the cellular pole (98). It should be added that PBP3 can occur in a dimeric form (187), like PBP1b (188). Dimerization of PBPs has been incorporated in the three-for-one model (Fig. 3).

Spatial distribution of mRNAs. In the foregoing, it has been assumed implicitly that divisome components are randomly positioned in the cytoplasm or in the cytoplasmic membrane before being assembled into a localized divisome. As an alternative, again taking *Drosophila* as the example (Fig. 12), mRNAs might be present at defined cellular locations. For example, in the case of cytoplasmic FtsZ, preferential cellular location of its gene or of its messengers would not occur. In contrast, at division, spatial rearrangement of the chromosome would direct the *ftsZ* gene to the cell center to provide for mRNAs at the spot. Is such a scenario likely? At a first glance, it is not. The cell size difference between *Drosophila* and *E. coli* is so huge that it is difficult to believe that diffusion will not distribute proteins in *E. coli* extremely rapidly. On the other hand, the importance of the structural organization should not be underestimated (Fig. 9). In fact, recent developments in microscopy such as the green-fluorescence technique (17) might allow these ideas to be tested. Such an approach should give us an idea about the degree of bacterial macromolecular organization as related to polarized processes.

Genotype and Phenotype

Introduction. First, I will summarize some notions which have been developed in the foregoing sections. (i) A cylindrical soap bubble is an analog for the shape of *E. coli* (79, 80). (ii) With respect to cell elongation and cell division at the peptidoglycan level, specificity resides in the respective enzymes being active and not in the chemical makeup of the sacculus. This argues in favor of physical forces in shaping the cellular form. (iii) The sacculus is not an independent entity. It is structurally connected to the cytoplasmic membrane via the transmembrane HMW PBPs. Many membrane proteins as such become inserted through a mechanism of cotranslational protein synthesis. Translation and transcription in turn go hand in hand, thus connecting (mobile?) DNA loops to the cytoplasmic membrane. (iv) *E. coli* does not have an eukaryote-like cytoskeleton. However, arguments have been presented to suggest that there is a cytoplasmic supportive structure composed of EF-Tu and FtsZ. (v) The structural connection between periplasm and cytoplasm at sites of cell elongation is quite different from the one at the site of cell division. This difference results from rearrangement of the macromolecular fabric, which is believed to be due, at least in part, to the partitioning status of the nucleoid. (vi) Various envelope domains have been proposed to exist: old poles, new poles, lateral wall next to the nucleoid, and lateral wall between nucleoid and pole. For each domain, the interaction between the cytoplasm and periplasm is supposedly different. (vii) As a consequence, each newborn cell is polarized from the outset. Polarization arises from polarization. (viii) Eubacteria as well as archaebacteria (without peptidoglycan) use the cell division protein FtsZ to carry out division. Although some prokaryotes can do without peptidoglycan, *E. coli* cannot. It should be borne in mind that the essential cell division gene *ftsI* encodes a penicillin-binding protein (PBP3).

Cell shape and *E. coli*. Depending on the growth conditions, *E. coli* varies in size; fast-growing cells are bigger than slowly growing ones (for a review, see reference 123). At all growth rates, its shape can be roughly approximated by a cylinder with hemispherical ends. However, cells differ in their aspect ratio: the mean cell length (L) divided by twice times the radius ($2R$) (185). Obviously, cells with a small aspect ratio are short while a relatively large part of their surface is occupied by polar caps. In contrast, long cells have quite a different surface proportion of side wall and polar cap material. In an earlier section, it was mentioned that PBP2 is involved in cell elongation whereas PBP3 participates in polar cap formation. Specific antibiotics against these PBPs (mecillinam and cephalexin, respectively) result in inhibition of peptidoglycan synthesis as related to cell shape (Fig. 16). That is, the higher the aspect ratio, the smaller

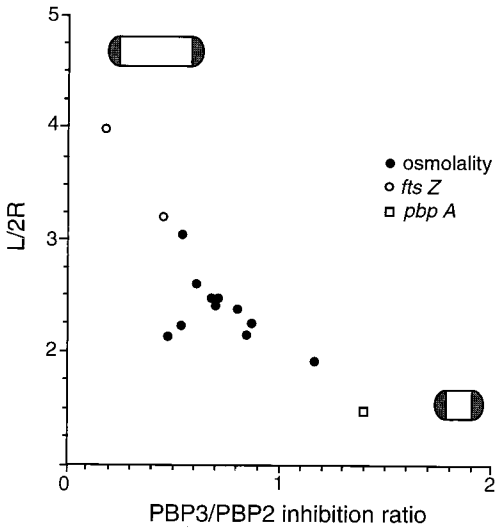

FIG. 16. Relation between cell shape of MC4100 *lysA* and the PBP3/PBP2 inhibition ratio. The average cell dimensions have been measured, and the length/width ratio (*L*/2*R*) has been calculated. Briefly, the different shapes were obtained by varying the osmolality of the medium and the temperature and by using *pbpA* (encoding PBP2) and *ftsZ* derivatives of MC4100 *lysA*. Further details can be found in the legend to Fig. 6 in reference 177. Each culture was probed for inhibition of [^{3}H]DAP incorporation by PBP3-specific cephalexin (10 μg/ml) and PBP2-specific mecillinam (2 μg/ml). The ratios of these inhibition percentages were taken as the PBP3/PBP2 ratio. Reprinted from reference 177 with permission of the publisher.

the PBP3/PBP2-inhibition ratio (122, 177). Clearly, the two PBPs play a role in maintaining cell shape.

Recently, it was suggested (67) that the sacculus as such would act as a template to be copied by a multienzyme complex (Fig. 5). However, this does not seem likely, because it is difficult to imagine how the aforementioned multienzyme complex could do more than produce glycan strands. It is still a big step from 30-nm glycan strands (Fig. 2) to an intact sacculus.

This brings us back to a question posed at the beginning of this review: where does gene expression end, and where does physics come in? It was also mentioned that there seems to be no clear-cut relation between chemical composition and cellular shape. So how should one understand the shape of *E. coli*?

Surface stress theory. A model which can account for the rod shape of *E. coli* by analogy to the "morphological" behavior of soap bubbles has been developed by Koch in recent years (82, 83; for reviews, see references 79 and 80). In *E. coli*, mass increase and hydrostatic pressure (22) take the place of the bubble blower. As outlined above, a cylindrical soap bubble can form between two fixed rings (Fig. 1), which are equivalent to the more or less rigid polar caps. The cylindrical shape becomes possible because the membrane of the soap bubble is fluid. According to Koch (80), four criteria must be fulfilled for stable cylindrical elongation. (i) The lateral wall has to enlarge according to a dispersive mode of insertion of building blocks. This has been demonstrated by electron microscopic autoradiography for the incorporation of [^{3}H]DAP into the peptidoglycan layer and by immunogold labeling for the insertion of lipoprotein and LamB in the outer membrane (for a review, see reference 122). (ii) The polar caps should be relatively rigid. Reanalysis of the autoradiographic data of Woldringh et al. (181) concerning [^{3}H]DAP incorporation has revealed that this is the case (83). The same result was obtained when cells that had incorporated D-Cys were chased into a D-Cys-free medium. Isolation of sacculi and labeling of D-Cys with immunogold after biotinylation revealed diluted label in the lateral wall and nondiluted label in the cell poles (33). (iii) The criterion of sufficient turgor pressure also seems to be met (78). (iv) The growing sacculus "must be one in which the mathematics for fluid membranes apply and not the mathematics for stresses in a static solid" (80). In other words, are the lateral sides of a monolayered sacculus also fluid? This is very likely. In situ during growth, fluidity is achieved by lytic and synthetic enzymes, which are active all over the lateral surface. "Sacculus fluidity" would thus depend on the proper balance between synthetic and lytic activities in relation to its growth. This suggests that PBP2 plays a central role in maintaining this balance. Therefore, it would seem that the "morphogene" *pbpA* codes for a gene product (PBP2) that contributes to the maintenance of sacculus fluidity. It does not seem to determine cell shape but to contribute to the production of a suitable structure for physical factors to act upon.

Cell division in *E. coli* could be explained if the surface tension (*T*) were lowered in the center of the cell. It has been calculated that a twofold change in *T* would be sufficient to cause invagination of the cell envelope (Fig. 17) (variable *T* model [80, 81]). In Fig. 17, it can also be seen that the extent of the decrease in *T* affects the shape of the constriction. As mentioned previously (118), impairment of PBP3 by mutation or by the PBP3-specific antibiotic furazlocillin leads to a constriction, similar in shape to that simulated by a 1.33- or 1.55-fold reduction in *T*. Interestingly, pointed polar caps have been observed in another temperature-sensitive PBP3 mutant (*pbpB*^{r1}) when grown at the nonpermissive temperature (155). Pointed polar caps might be expected if the reduction in *T* is 1.75 (Fig. 17). These striking correlations indicate but do not yet prove that the activity of PBP3 is influenced by surface tension. In the case of long and smooth *ftsZ* filaments, *T* obviously is not reduced at potential division sites. Still, it is difficult to understand what "soap bubble property" can account for their morphological stability. To test the variable *T* model, one would like to measure *T* at the cell center, which unfortunately is not possible at present.

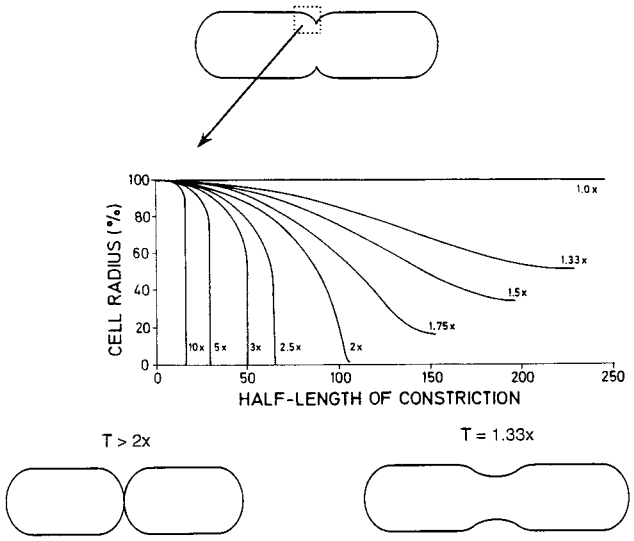

FIG. 17. Extent of constriction as a function of the local fold decrease in surface tension, *T*. The square in the upper figure refers to the lower graph. If the fold decrease of *T* is greater than 2, division can take place (lower left figure). In the case of, for instance, a 1.33- or 1.5-fold decrease in *T*, division cannot proceed and cells with so-called blunt constrictions arise (lower right figure). This situation applies after impairment of PBP3. Modified from reference 81 with permission of the publisher.

Hydrostatic pressure has also been invoked in relation to tip growth in fungi, although in some organisms no hydrostatic pressure seemed to be present (58). This is a puzzling situation, which could reflect the technical difficulty in measuring hydrostatic pressure or our limited understanding of a morphogenetic process (see also reference 175).

Cell shape and the interaction of cytoplasm and periplasm. Does the macromolecular fabric of the envelope and underlying cytoplasm play a role in maintaining the rod shape? It has been suggested in relation to the three-for-one model that existing template strands determine the length of the glycan chains coming in, thus also determining the cell diameter (66). However, as pointed out by Koch (80), the stress-bearing existing fabric will have a quite different spatial organization from the incoming unstressed polymers, making the idea of a glycan chain yardstick perhaps unlikely. What about the *E. coli* cytoplasm? Clearly, the ease with which spheroplasts are formed after the sacculus is degraded by lysozyme precludes a dominant role for a cytoskeleton-like structure in maintaining the rod shape. It might be speculated that the hypothetical cytoplasmic supportive structure helps to subdivide the cytoplasm into discrete functional domains. This would allow the safe transcription of (for instance) the *pbpA* gene and proper insertion and activation of its gene product (PBP2) in its membraneous environment, suitable for peptidoglycan synthesis. Perhaps this cytostructural context forms the material link between genotype and phenotype (at least in *E. coli* [57]). As has already been discussed extensively above, the relevance of cytoplasmic organization (polymerization of FtsZ and divisome assembly) is much more apparent in the case of cell division.

OUTLOOK

The shape of *E. coli* is strikingly simple compared to those of higher eukaryotes. Outwardly, it resembles a lower eukaryote like *S. pombe*. However, the latter not only is larger (7 to 13 μm long and 2 to 3 μm in diameter versus 1.5 to 5.5 μm long and 0.5 to 1.0 μm in diameter) but also harbors membrane-bound organelles and a cytoskeleton. Shaping of *S. pombe* will certainly require more gene products. Physical principles underly morphogenesis, as has been expressed most clearly by Koch (reference 78 and references therein). Such principles "act" on a chemical substrate which changes during the division cycle of a cell (chemodifferentiation). Clearly, morphogenes (not to be confused with morphogens) do not exist (57). Rather, the regulation of gene expression in response to external and internal signals, the synthesis of gene products and of a dynamic macromolecular fabric, and the creation of turgor through growth of cellular mass (22) all contribute in providing the chemical substrate referred to above. There are two clear limitations which hamper the further understanding of bacterial morphogenesis: (i) knowledge about the temporal regulation of the cellular organization of the macromolecular fabric and (ii) the direct measurement of physical parameters in a small cell such as *E. coli.*

Nevertheless, one might distinguish three approaches to further our understanding of morphogenesis. (i) The first one involves the targeting and interaction of proteins. The technologies for such studies are available and are already widely used, not only in *E. coli* but particularly in yeast and animal cells. The combination of molecular genetics, protein engineering, and fluorescence microscopy is likely to ensure progress in solving the structure of the divisome in the not too distant future. In particular, the availability of the complete genomic sequence and the use of green fluorescent protein to study the dynamics of protein localization in living cells will be important tools in this effort. (ii) No direct approach to measure surface tension in different envelope domains seems possible at present. However, an alternative would be to attempt to purify these domains and to determine their protein and lipid composition. This might well work if one could isolate inside-out vesicles with bound FtsZ. Then biophysics and physical chemistry could be applied to reconstituted membrane vesicles with defined composition and contents to better grasp the effects of physical factors on shape. (iii) More difficult to investigate will be the spatial arrangement of small molecules, like water, and of proteins in the cytoplasm (Fig. 9b). Relevant ideas are generally based on (plausible) extrapolation from known properties of the cytoplasmic constituents (72). Because cytoplasmic organization is lost upon cell disruption, techniques that can detect molecular behavior in situ are required. Thus, spectroscopic techniques combined with three-dimensional microscopy, the latter giving the spatial cellular coordinates of the molecule involved, are now required. The small size and structural "simplicity" of *E. coli* will certainly be an advantage for such approaches, making *E. coli* a model organism for the study of living matter.

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